Detection of *Escherichia coli* O157:H7 using a surface plasmon resonance biosensor

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The BIAcore biosensor was used to detect binding of *Escherichia coli* O157:H7 with specific antibodies. Immobilized Protein A or Protein G captured antibodies which in turn bound to the bacteria. Alternatively, immobilized antibody captured the *E. coli* O157:H7 and the bacteria were further probed by a second antibody which enhanced the signal. The regenerated sensor surfaces were used for at least 50 separate analyses. The surface plasmon resonance biosensor has potential for use in rapid, real-time detection and identification of bacteria, and to study the interaction of organisms with different antisera or other molecular species.

Introduction

Methods for detection of food-borne pathogens have evolved from conventional culture techniques to more rapid and specific immunological assays and methods based on nucleic acid probes and the polymerase chain reaction. Many of these methods, however, are still laborious, require at least 24 to 48 h to obtain final results, and may require significant training to perform. In recent years, various types of biosensors have been developed which allow direct measurement of chemical species in biological samples (Kress-Rogers, 1997). However, reports on the use of biosensors for detection of food-borne pathogens are lacking.

Biosensors are analytical instruments possessing a capturing molecule as a reactive surface in close proximity to a transducer which converts the binding of an analyte to the capturing molecule (ligand) into a measurable signal. The BIAcore instrument is a biosensor which employs real-time biomolecular interaction analysis (BIA) to monitor biological processes at the molecular level. A signal is generated through the optical phenomenon of surface plasmon resonance (SPR) monitored by a fixed array of light sensitive detectors. The instrument is equipped with sensor chip technology consisting of a gold film on a glass backing with a surface matrix of carboxymethyl dextran attached to the gold film. The interactant (ligand) is covalently linked by the user to the dextran layer on the surface of the sensor chip. The capture of a molecule

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(analyte) results in a mass increase producing a change in the refractive index in close proximity to the sensor surface. SPR converts the refractive index changes into optical signals expressed as resonance units (RU). Continuous monitoring of changes in RU as a function of time is displayed as a sensorgram. Thus the progress of association of ligand and analyte, dissociation, and regeneration of the sensor chip surface (removal of bound analyte) are recorded. An integrated microfluidic cartridge (IFC) controls delivery of the sample, buffer and eluant through designated channels to the sensor surface. The instrument software controls the operation of the IFC. Data point readings are taken between sample injections while constant buffer flow is operating. The BIAcore biosensor enables automated real-time monitoring of biomolecular interactions, does not require labeling of the interactants, and can allow determination of kinetic rate constants.

Applications of BIAcore technology include assessment of antigen-antibody interactions, epitope mapping, determination of the affinity and kinetics of interactions, its use for determining concentrations of analytes in solution, for examining ligand-receptor interactions, and also for performing nucleic acid studies and for screening foodstuffs or other materials for pesticide, antibiotic or drug residues (Fägerstam *et al.*, 1992; Karlsson *et al.*, 1994; Medina, 1997; Mellgren *et al.*, 1996; McNally *et al.*, 1995; Minunni and Mascini, 1993; Natsume *et al.*, 1994; Nilsson *et al.*, 1995; Pfaff *et al.*, 1994). With suitable ligands on the sensor chip surface, binding of whole cells such as bacteria or human blood cells can be studied (BIAtechnology Note 103, 1994). In the present study, the BIAcore biosensor was employed for detection of viable *Escherichia*

coli O157:H7 using antibodies reactive against this pathogen.

Materials and methods
Bacteria
The bacteria used in this study were the following: *E. coli* O157:H7 B1409 and *E. coli* O157:H7 933 (Centers for Disease Control and Prevention, Atlanta, GA), *E. coli* O157:H7 ATCC 43890, *Salmonella typhimurium* ATCC 14028, *E. coli* O157:H7 93–569 (Food Safety and Inspection Service, Beltsville, MD), *E. coli* O103:H2 87.1368 (*E. coli* Reference Center, University Park, PA), and *Yersinia entercolitica* GER O:3 (Food and Drug Administration, Seattle, WA). Bacteria were grown in brain heart infusion broth at 37°C for 16 h with aeration. Aliquots of the cultures (1 ml) were then centrifuged at 16,000 $\times$ *g* for 2 min and the cell pellet was suspended in 1 ml of HBS buffer [10 mM HEPES, pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.005% BIAcore surfactant P20 (Biacore, Inc., Piscataway, NJ)].

Reagents and instrumentation
These studies were performed on the BIAcore instrument (Biacore, Inc.) equipped with BIAlogue command software. Monoclonal antibody 8–9H (IgG2a subtype) reactive against *E. coli* O157:H7 was kindly provided by Dr. Rebecca Durham at Organon Teknika, Rockville, MD. Unlabeled goat anti-*Escherichia coli* O157:H7 affinity-purified polyclonal antibody and goat anti-*Salmonella* (CSA-1) were purchased from Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD. Other materials included: Protein A (purified from *Staphylococcus aureus*; Sigma, St. Louis, MO), purified recombinant Protein G (Pierce, Rockford, IL), sensor chip CM5, and the amine coupling kit containing *N*-ethyl-*N'*-(dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS) and ethanolamine (Biacore, Inc.). Additional chemicals used were of reagent grade.

Immobilization
Ligands were immobilized onto flow cells of sensor chip CM5 by the amine coupling method (O'Shannessy *et al.*, 1992). Briefly, with this coupling method, the carboxymethylated dextran layer on the sensor surface is activated with an EDC/NHS mixture to produce NHS-esters which can react with ligands containing primary amino groups. After immobilization of the ligand, unreacted NHS-esters are deactivated following injection of a large excess of ethanolamine hydrochloride. Noncovalently bound ligand is then washed from the surface with 100 mM HCl. Ligands bound to the sensor chip flow cells included: Protein A (300 μg/ml in 10 mM NaOAc, pH 4.5), Protein G (150 μg/ml in 10 mM NaOAc, pH 4.5), polyclonal affinity-purified antibody (100 μg/ml in 10 mM NaOAc, pH 4.5), and monoclonal antibody 8–9H (ascites, 100 μg/ml in 10 mM NaOAc, pH 4.5). HBS, pH 7.4, was used as the 'running' (wash) buffer between injections.

Detection of bacteria
Following immobilization of Protein A or Protein G on the sensor chip surface, either monoclonal or polyclonal antibodies against *E. coli* O157:H7 were applied at concentrations of 200 μg/ml or 50 μg/ml (diluted in HBS, pH 5.0 or 7.4), respectively, at a flow rate of 3 μl/min for a total of a 10-min injection. The bacterial suspension (in HBS, pH 5.0 or 7.4) was then injected across the surface also at a flow rate of 3 μl/min for a total of a 10 min injection and allowed to interact with the bound antibodies. Between injections, the surface was washed with HBS (pH 5.0 or 7.4) to remove unbound material. The sensor chip surface was regenerated with two 1 min pulses of 6 M guanidine-HCl, pH 1.0.

In attempts to increase resonance signals a 'sandwich' assay format was programmed. Using an automated sequential injection and wash method, the bacterial suspensions were injected over surfaces with immobilized monoclonal or polyclonal *E. coli* O157:H7 antibodies (first antibody). Then either monoclonal (200 μg/ml) or polyclonal antibody (50 μg/ml) (second antibody) in HBS, pH 7.4 was injected. Between injections of the bacteria and antibodies, the sensor surface was washed with HBS to remove excess unbound material. Both the bacterial suspension and the second antibody were injected for 10 min at 3 μl/min. As in the previous assay, the sensor surface was regenerated with 2 one minute injections of 6M guanidine-HCl, pH 1.0.

To determine the limit of detection, 10-fold serial dilutions, prepared in HBS, of *E. coli* O157:H7 cultures were injected over a monoclonal antibody surface. Following a wash with HBS, polyclonal antibody diluted in HBS to a concentration of 50 μg/ml was injected for 10 min at a flow rate of 3 μl/min.

To visualize bacteria bound to antibody on the sensor chip surface, various levels of *E. coli* O157:H7 were injected over a surface to which monoclonal antibody was bound. The surface was washed with HBS. The sensor chip was removed and the surface was then examined by optical microscopy for the presence of bacteria and photographed.

Results and discussion
In the present study, various test systems were used to monitor binding of whole bacterial cells to the BIAcore
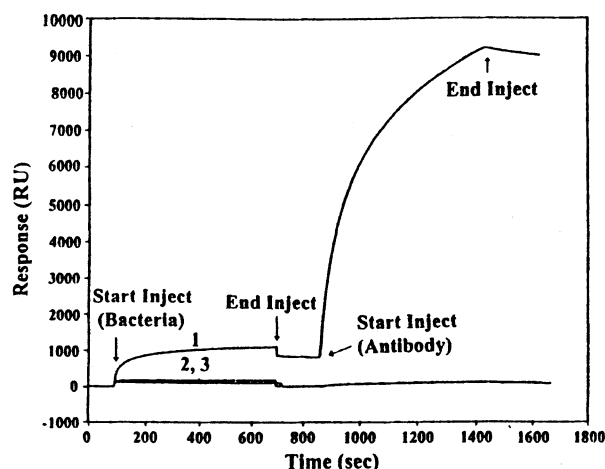
Figure 1 Overlay plots of sensorgrams showing the interaction of monoclonal antibody 8–9H (ligand) with *E. coli* O157:H7 (1), *S. typhimurium* (2), and *Y. enterocolitica* (3) followed by injection of polyclonal antibody at 50 μg/ml. The bacteria were injected at about 5 × 10⁹ cfu/ml.

sensor surface in efforts to develop a real-time assay for detection of *E. coli* O157:H7. Binding of *E. coli* O157:H7 B1409 at about 5 × 10⁹ cfu/ml to monoclonal antibody 8–9H immobilized on the sensor surface generated a response signal of 804 RU (Fig. 1). A 10 min pulse of *S. typhimurium* or *Y. enterocolitica* suspensions (ca 5 × 10⁹ cfu/ml) did not generate a notable response (7 and 17 RU, respectively). These results indicate that the antibody was specific against *E. coli* O157 and had insignificant cross-reactivity with the other bacteria tested. Injection of whole bacteria through the instrument's liquid handling system did not cause disturbances in the flow system.
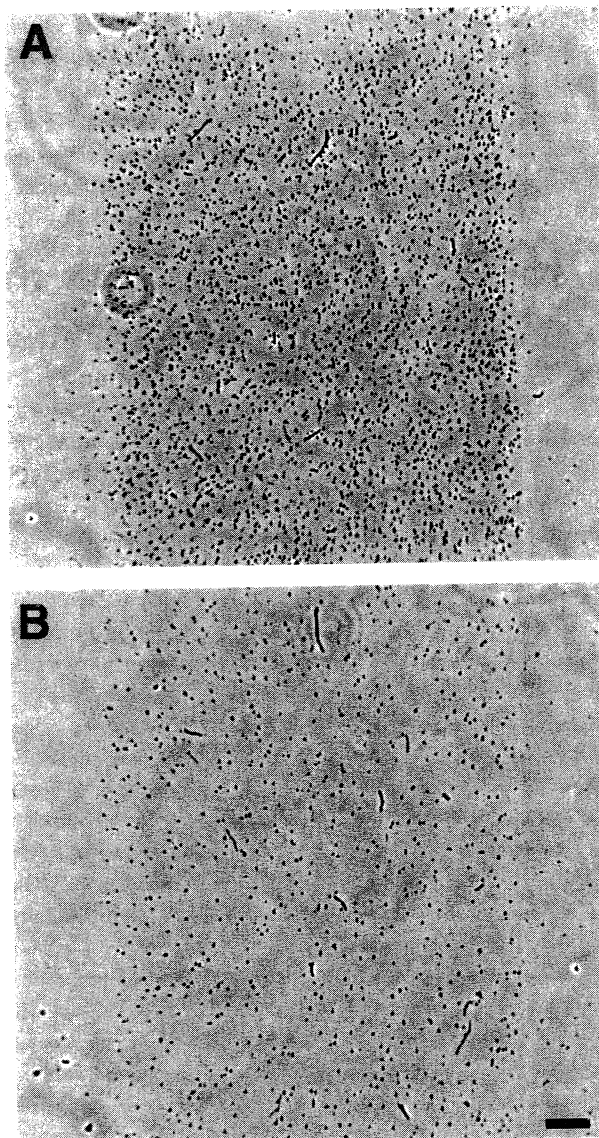
In order to enhance the signal obtained with *E. coli* O157:H7 and to confirm the specificity of the binding, injection of bacteria was followed by a 10 min pulse of polyclonal antibody. A signal of 8153 RU was obtained with surfaces to which *E. coli* O157:H7 bound (Fig. 1). Since *S. typhimurium* and *Y. enterocolitica* did not bind to the monoclonal antibody, no response with polyclonal antibody reactive against *E. coli* O157:H7 was obtained. Other first and second antibody combinations were tested including monclonal antibody ligand followed by monoclonal antibody as the second antibody, polyclonal antibody followed by polyclonal antibody and polyclonal antibody followed by monoclonal antibody. With every combination, signal was enhanced reproducibly with injection of the second antibody. Generally, the combination of monoclonal antibody as the ligand followed by polyclonal antibody produced the best response with *E. coli* O157:H7. Three other *E. coli* O157:H7 strains (933, 93–569 and ATCC 43890) were tested and similar results were obtained as with strain

B1409 while *E. coli* O103:H2 did not generate a notable signal (data not shown). To further confirm the specificity of the assay for *E. coli* O157:H7, antibody against *Salmonella* species was injected as the second antibody following a 10 min pulse of the bacterial suspensions over a monoclonal antibody 8–9H surface. No enhanced response was obtained with *E. coli* O157:H7 and since no remarkable binding of *S. typhimurium* or *Y. enterocolitica* occurred with immobilized monoclonal antibody, a response signal was not generated with injection of antibody reactive against *Salmonella*.

The detection limit of the assay was ca 5–7 × 10⁷ cfu/ml injecting a total of 30 μl (3 μl/min for 10 min, therefore, 1.7–2.1 × 10⁶ cfu injected). Generation of significant binding responses following injection of *Staphylococcus aureus* over surfaces with immobilized human IgG (through binding of Protein A molecules on the cell membrane to IgG) and binding of erythrocytes to immobilized concanavalin required injections of 5 × 10¹² cfu/ml of *S. aureus* and 4 × 10⁹ erythrocytes/ml (BIAtechnology Note 103, 1994). RU's obtained with injection of *E. coli* O157:H7 suspensions were rather low, therefore, to determine the level of bacteria bound to the sensor surface, the sensor chip was examined by optical microscopy. Bacteria were visible and evenly distributed over the entire flow cell of the sensor chip when injected at a concentration of 2 × 10⁹ cfu/ml (Fig. 2B) generating a signal of 403 RU. When injected at a level of 1 × 10¹⁰ cfu/ml, higher levels of bacteria bound to the surface (Fig. 2A) and a signal of 876 RU was obtained.
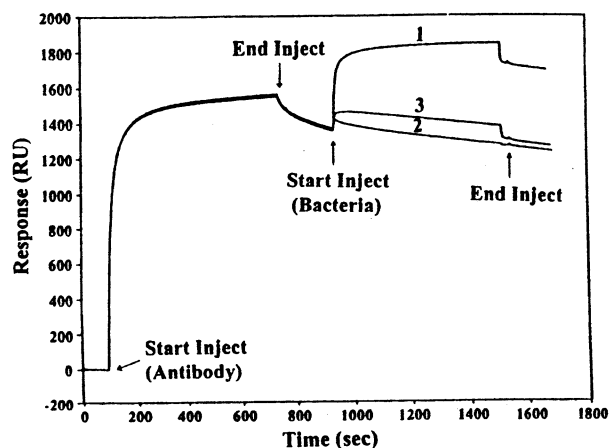
Several factors should be taken into consideration when monitoring interactions of whole cells using the BIAcore. The instrument generates a response with changes in the refractive index of the solution close to the gold film on the sensor surface. The effective penetration depth of the evanescent wave which arises under conditions of surface plasmon resonance is 0.3 μm. Therefore, refractive index changes occurring at distances less than 0.3 μm from the surface will cause a signal change. The bacteria probably do not penetrate the dextran layer (0.1 μm thick) which coats the gold surface. Thus, when bacteria bind to ligands bound to the dextran surface, only a small portion of the bacterial cell which is close to the sensor surface contributes to producing a response signal. Furthermore, the BIAcore detection system measures an average SPR angle over an area of approximately 0.25 mm² of the sensor surface. Since bacterial cells are large and do not evenly cover an area measured, the response signal is decreased.

When antibodies are coupled to the sensor surface, it is probable that they are bound in different orientations,

Figure 3 Overlay plots of sensorgrams showing the interaction of Protein A (ligand) with monoclonal antibody 8–9H at a concentration of 200 μg/ml followed by injection of the bacterial suspensions (*E. coli* O157:H7 (1), *S. typhimurium* (2) and *Y. enterocolitica* (3); ca 5 × 10⁹ cfu/ml).

Figure 2 Binding of *E. coli* O157:H7 to ligand (monoclonal antibody) on the sensor chip surface. The concentrations of injected bacterial suspensions were ca 1 × 10¹⁰ cfu/ml (A) and 2 × 10⁹ cfu/ml (B). The bacteria were injected for 10 min at a flow rate of 3 μl/min. Marker bar = 50 μm.

some of which may not allow binding of antigen to the antibody binding site. To ensure that antibody was bound in the proper orientation, a second assay format was designed. Protein A and Protein G were immobilized onto the sensor surface and used to capture the F_c portions of the monoclonal or polyclonal antibodies which in turn captured the bacteria through the binding sites. When the assay was performed using HBS at pH 7.4 and a Protein A sensor surface, injection of monoclonal antibody 8–9H at a concentration of 200 μg/ml generated an RU of approx-

imately 1400 (Fig. 3). The signals obtained following interaction of the bacteria (5 × 10⁹ cfu/ml) with the protein A-bound antibody were 326, −144 and −114 RU with *E. coli* O157:H7, *S. typhimurium* and *Y. enterocolitica*, respectively. Interestingly, this assay format did not appear to enhance the capture of *E. coli* O157:H7 or enhance the signal generated compared to use of the assay format in which antibody was directly immobilized onto the sensor surface (804 RU) (Fig. 1). Apparently, more antibody-binding sites are accessible when antibody is immobilized directly on the dextran surface. The highest signal was generated with the assay format in which antibody was immobilized on the sensor surface followed by binding of bacteria then of second antibody.

Both monoclonal and polyclonal antibodies bound at higher levels to Protein A and Protein G, in particular to Protein G, when they were prepared and tested in HBS, pH 5, than when tested in HBS, pH 7.4 (Table 1). However, the level of bacteria which subsequently bound to either monoclonal or polyclonal antibody was apparently not influenced by the pH of HBS. Generally, more bacteria bound to antibody when higher levels of anitibody had bound to Protein A or G. Medina and Palumbo (1996) also found that optimum binding of sheep IgG to Protein G occurred using HBS, pH 5. They found that binding of the sheep IgG to Protein G was 7-fold higher than to Protein A as has also been reported by others (Newman *et al.*, 1997). The binding capacities of Protein A and G with antibodies derived from different animal species vary. Under the binding conditions used in the present study, both monoclonal (from mouse) and polyclonal (from goat)

Table 1 Interactions of immobilized Protein A and Protein G with anti-*E. coli* O157:H7 antibodies and of protein A/G-bound antibody with *E. coli* O157:H7 tested at pH 5 and pH 7.4.

| | | Resonance units generated with interactions[a] | | | |
| | | pH 5 | | pH 7.4 | |
Ligand	Antibody[b]	Antibody	E. coli O157:H7[c]	Antibody	E. coli O157:H7
Protein A	Polyclonal	355 (28)	170 (13)	381 (10)	63 (11)
Protein G	Polyclonal	2974 (189)	318 (9)	2716 (45)	543 (40)
Protein A	Monoclonal	1525 (165)	148 (42)	1357 (51)	301 (25)
Protein G	Monoclonal	4279 (41)	568 (44)	1463 (39)	287 (17)

[a] A change in the resonance angle or signal is expressed in resonance units. Standard deviations of triplicate determinations are given in parenthesis.
[b] Monoclonal antibody 8–9H and polyclonal antibody were diluted in HBS at pH 5 or 7.4 (200 µg/ml).
[c] Bacterial suspensions were suspended in HBS pH 5 or pH 7.4 at about 5×10^9 cfu/ml.

antibodies bound at higher levels to Protein G, than to Protein A, especially at pH 5. According to Newman *et al.* (1997), the binding capacities of mouse IgG, subtype 2a to Protein A and G and of goat IgG to Protein A and G are similar. However, binding is influenced by reaction conditions, particularly pH.

Antibody and Protein A and G surfaces were readily 'regenerated'. During regeneration, only layers of antibody and/or antigen are removed, leaving the covalently immobilized ligand undisturbed. In the present study, the sensor chip surface could be employed for at least 50 different analyses without observing significant differences in binding of antibody to Protein A or G or of bacteria to immobilized antibody.

With improved BIAcore system instruments (models 1000 or 2000), new sensor chip designs, and with further optimization of the system, sensitivity for detecting whole cells should be considerably enhanced. If appropriate antibodies are used, the biosensor can be employed for rapid identification of bacterial colonies recovered from selective agar growth medium and can also potentially be used to detect target organisms in enrichment cultures of foods or other types of samples. For small analytes, the lower limit of detection of the BIAcore system used in the present study is approximately 10 pg analyte/mm^2 (Jönsson *et al.*, 1991). A number of reports have shown that the BIAcore biosensor can be employed for monitoring levels of antibodies, hormones, veterinary drugs and antibiotics in foods and other materials even using crude samples (BIAtechnology Note 103, 1994; Mellgren *et al.*, 1996; Newman *et al.*, 1997). Medina *et al.* (1997) optimized the binding interactions of *E. coli* O157:H7 antibodies with immobilized bacteria and further studied the binding of extracellular matrix macromolecules with the immobilized cells (Medina and Fratamico, 1998). The present study shows

that BIAcore system can be used to investigate the binding interactions of *E. coli* O157:H7 to specific antibodies and can potentially also be used to study interactions with other molecular species such as cell receptors or extracellular matrix proteins. The use of a 'sandwich' assay in which binding of bacteria is followed by injection of antibody can markedly increase the signal generated and confirm the specificity of the interaction.

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